

BIOCONTROL POTENTIALITY AND PLANT GROWTH PROMOTING ACTIVITY OF *PSEUDOMONAS FLUORESCENS* - A REVIEW

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ABSTRACT

Fluorescent Pseudomonads belong to plant Growth Promoting Rhizobacteria (PGPR) the important major group of bacteria that play a major role in the plant growth promotion, induced systemic resistance, biological control of pathogens. Many strains of Pseudomonas fluorescens show potential for biological control of phytopathogens especially root pathogens. P. Fluorescens is well characterized for their ability to produce antimicrobial compounds, including 2, 4-diacetylphloroglucinol (DAPG), phenazines, hydrogen cyanide and surfactants. The present review deals with different mechanisms of biocontrol of plant pathogens by Pseudomonas fluorescens.

KEYWORDS: 4-Diacetylphloroglucinol (DAPG), Phenazines, Hydrogen Cyanide & Surfactants

Received: Oct 02, 2016; **Accepted:** Oct 18, 2016; **Published:** Oct 25, 2016; **Paper Id.:** IJASRDEC201624

INTRODUCTION

Biological control is a potential non-chemical means for plant disease management by reducing the harmful effects of a parasite or pathogen through the use of other living entities. Plant growth promoting rhizobacteria are the group of soil bacteria inhabiting around/on the root surface and are involved in promoting plant growth and development directly or indirectly via production and secretion of various secondary metabolites in the vicinity of rhizosphere. Direct promotion of plant growth by PGPR is take place by production of phytohormones like auxin and cytokinin and indirect promotion of plant growth by reducing the harmful effects of plant pathogens by various biocontrol mechanisms. Among the PGPR, fluorescent Pseudomonads are the most exploited bacteria for biological control of soil-borne and foliar plant pathogens. In the past three decades, numerous strains of fluorescent Pseudomonads have been isolated from the rhizosphere soil and plant roots by several workers and their biocontrol activity against soil-borne and foliar pathogens were reported (Vivekananthan *et al.*, 2004). Fluorescent Pseudomonads are non-pathogenic rhizobacteria which suppress the soil-borne pathogens through rhizosphere colonization, antibiosis, iron chelation by siderophore production and ISR. *Pseudomonas fluorescens* is one of the important PGPR and it has many characteristic features like able to grow rapidly *invitro* and can be mass produced easily, able to utilize exudates of seed and root, effectively colonize and multiply in the rhizosphere and spermosphere environments and also has the ability to produce wide spectrum of bioactive metabolites and compete aggressively with other microorganisms so that it can effectively adapt to different environmental stresses and inexpensive (Weller, 2007). Here in this paper we reviewed different biocontrol mechanisms against different plant pathogens.

ANTIBIOSIS

Antibiotics are defined as heterogeneous group of low-molecular-weight organic compounds that are deleterious or harmful to the growth or metabolic activities of other microorganisms. The antibiotics were more effective in suppressing the growth of target pathogen *in vitro* and *in situ*. The production of one or more antibiotics is the most important mechanism of plant growth promoting rhizobacteria which facilitates the antagonistic against many phytopathogens (Glick *et al.*, 2007). *Pseudomonas fluorescens* 2-79 and 30-84 produce phenazines against *Gaeumannomyces graminis* var. *tritici* (Thomashow *et al.*, 1990). *Pseudomonas fluorescens* Fl 13 produces 2, 4-diacetyl-phloroglucinol against *Pythium* spp. (Shanahan *et al.*, 1992). The involvement of antibiotics in disease suppression was first demonstrated by testing antagonistic activity of antibiotic 2, 4- diacetylphloroglucinol (DAPG) against take-all decline of wheat (Weller *et al.*, 2002). Occasionally siderophores behave as antibiotics though it was a part of primary metabolism (because iron is an essential element). *In vitro*, these antibiotics inhibit fungal pathogens, but they can also be active against many bacteria and, in some cases, against higher organisms.

Six classes of antibiotic compounds are better associated with biocontrol of plant diseases: Phloroglucinol, phenazines, pyrrolnitrin, pyoluteorin, cyclic lipopeptides and hydrogencyanide (Haas and Defago, 2005). The important antibiotic 2,4-diacetylphloroglucinol (DAPG) produced by pseudomonads has effectively cause membrane damage to *Pythium* spp. and particularly has inhibitory action against zoospores of *Pythium* spp. (de souza *et al.*, 2003). Pseudomonads are potential biocontrol agents, that showing competitive interactions with other micro organisms including fungi, bacteria, protozoa and nematodes by producing lipopeptide biosurfactants (de Bruijn *et al.*, 2007; Raaijmakers *et al.*, 2010). The *Pseudomonas fluorescens* BL915 strain has inhibitory action against damping-off causing *Rhizoctonia solani* in cotton plants by producing pyrrolnitrin antibiotic (Hill *et al.*, 1994). Pseudomonads has the capacity to produce phenazine antibiotic, showing antagonistic activity against *Fusarium oxysporum* and *Gaeumannomyces graminis* (Chin-Awoeng *et al.*, 2003) and these phenazine antibiotic plays a role in mobilization of iron in soils at neutral soil pH, where iron is present in insoluble ferric form and this was experimentally proven with *Pseudomonas chlororaphis* PCL1391 strain isolated from rhizosphere of tomato plants (Hernandez *et al.*, 2004; Haas and Defago, 2005).

The relative importance of antibiotic production by bacterial bioagents has been demonstrated, where one or more genes responsible for biosynthesis of the antibiotics have been manipulated. For example, mutant strains incapable of producing phenazines (Thomashow and Weller 1988) or phloroglucinols (Keel *et al.* 1992, Fenton *et al.* 1992) have been shown to be equally capable of colonizing the rhizosphere but much less capable of suppressing soilborne root diseases than the corresponding wild-types and the capacity to produce multiple classes of antibiotics, that differentially inhibit different plant pathogens, is likely to enhance biological control effectively. *Pseudomonas putida* WCS358r strains were shown the capacity of phenazine and DAPG production by genetic engineering with corresponding genes and showed improved capacity to suppress or inhibit plant diseases in wheat crop (Glandorf *et al.*, 2001; Bakker *et al.*, 2002). Imran *et al.*, 2006 reviewed the role of cyanide in controlling root knot disease of tomato.

COMPETITION

The rhizosphere is the area surrounding the root and it is a narrow region of soil that is directly influenced by root secretions, associated with soil microorganisms, where rhizoplane is the area that covering external surface roots together with closely adhering soil particles. The rhizosphere and rhizoplane are significant nutrient sinks, attracting diverse microorganisms including plant pathogens. Competition for these nutrients is the important fundamental mechanisms by

which pseudomonads suppress the phytopathogens by depriving from nutrients (Duffy 2001). Competition phenomenon was difficult to be proven directly, much indirect evidence suggests that competition between pathogens and non-pathogens for nutrient resources is important for restricting disease incidence and severity. Soilborne pathogens like *Fusarium* and *Pythium* are more susceptible to competition by other soil and plant associated microorganisms than other pathogens like *Rhizoctonia* and *colletotrichum*, where former one infecting through mycelia contact and later one germinate directly on plant surfaces which they invade through appressoria and infection pegs. Rhizosphere or phyllosphere biocontrol agents generally protect the plant by rapid colonization, thus consuming completely the limited available substrates so that none is left for pathogens to grow. For example Suppression of *Pythium ultimum* by bacterial bioagent *Enterobacter cloacae* was reported by competing each other for nutrients in spermosphere (van Dijk and Nelson 2000; Kageyama and Nelson, 2003).

Competition for essential micronutrients, such as iron has reported. Iron is extremely limited in the highly oxidized soil as it is insoluble ferric form in water a pH 7.5 and the concentration of available iron may be as low as 10^{-18} M. This concentration is insufficient to support the growth of microorganisms. To overcome or survive this iron limited conditions microorganisms able to secrete iron chelating compounds called siderophores. Importance of siderophores production as a mechanism of biocontrol was first reported by controlling soft rot bacteria *Erwinia carotovora* with *Pseudomonas fluorescens* strains (Kloepper *et al.*, 1980). Fluorescent Pseudomonad species, viz., *P. fluorescens*, *P. stutzeri*, and *P. putida*, produce siderophore named as pseudonoline (Lewis *et al.* 2000 ; Mercado-Blanco *et al.* 2001)

INDUCED SYSTEMIC RESISTANCE

Van Peer *et al.*, 1991 reported that *Pseudomonas* strain WCS417 induced resistance in carnation against wilt caused by *Fusarium oxysporum f. sp. dianthi* when the roots were inoculated with bacteria 1 week prior to stem inoculation with the pathogen. This strain was isolated from the wheat rhizosphere and also promoted the growth of several crops. Subsequently, strains WCS417 and WCS374 were shown to induce resistance in radish against *F. oxysporum f. sp. raphani* and other pathogens (Hoffland *et al.*, 1996). The O-antigenic side chain of the lipopolysaccharide, present on the outer membrane of strains WCS374 and WCS417, appeared to be the determinant responsible for the induction of resistance in radish (Leeman *et al.*, 1995). Antibiotics also play key role in inducing resistance in plants and the importance of DAPG production in ISR was further supported by observations that mutants which were unable to produce DAPG, not having the capacity to induce resistance and finally concluded that antibiotics are major components for triggering ISR (Weller *et al.*, 2004). Under iron limited conditions several Pseudomonads produce salicylic acid which in turn play a major role in SA-dependent signal transduction pathway. However, ISR by SA producing *Pseudomonas* strains does not depend on SA but it is frequently observed in Siderophores (Ran *et al.*, 2005).

PHYTOHORMONES

Indole-3-Acetic Acid

Indole-3-acetic acid is important member of phytohormone group and it regulates plant development activities like organogenesis, cellular responses like cell expansion, cell division and differentiation and tropical responses (Sivasakthi *et al.* 2014). The *Pseudomonas* isolates producing IAA have stimulatory effect on the plant growth. When the sweet potato crop is inoculated with the isolates capable of IAA production significantly increases the plant growth by the increased uptake of N, P, K, Ca and Mg (Farzana and Radizah, 2005). Increase in root length as well as the number of secondary roots in young seedlings through IAA production by microorganisms increases the chances of survival of seedlings due to enhanced capacity to anchor to the soil and absorb water and nutrients from the surroundings

(Patten and Glick 2002). In IAA-producing bacteria, L-tryptophan dependent auxin production was observed and reported to increase the grain yield and the number of branches (Asghar *et al.* 2002, 2004).

Cytokinins

Cytokinins promote cell divisions, cell enlargement, and tissue expansion and are believed to be the signals for mediation of environmental stress from roots to shoots. *P. fluorescens* can produce cytokinins as reported by Garcia *et al.* (2001).

P. syringae infection was efficiently controlled by *Pseudomonas fluorescens* G20-18 in Arabidopsis by allowing maintenance of tissue integrity and increased biomass yield (Grobkensky *et al.*, 2016)

Aminocyclopropane-1- Carboxylate (ACC) Deaminase

The stress hormone ethylene is the only gaseous phytohormone and produced upon physical or chemical to the plants which causes inhibition of plant root growth. Shah *et al.* (1998) reported that insertion of ACC deaminase gene within *Pseudomonas spp.* aided bacteria with capacity to produce ACC deaminase enzyme and thereby release stress which in turn elongates seedling roots. *Pseudomonas* strains having capacity to produce ACC deaminase enzyme were reported to promote plant growth under stressful condition such as flood (Grichko and Glick 2001).

CONCLUSIONS

Wang *et al.*, 2000 reported enhanced protection ability of *Pseudomonas fluorescens* strain CHA0 against *Pythium* damping-off in cucumber and soft rot causing *Erwinia* in potato by introducing ACC deaminase genes into *Pseudomonas fluorescens* strain CHA0. Saravanakumar, 2007 compared the ACC deaminase activity in *Pseudomonas fluorescens* strain TDK1 by treating groundnut plants under saline conditions and found that enhanced saline resistance with ACC deaminase gene while no resistance is observed in groundnut plants treated with *Pseudomonas* strain not having ACC deaminase gene activity.

The gene 1-aminocyclopropane-1-carboxylate (ACC) deaminase (AcdS) was cloned from *Pseudomonas fluorescens*, which has the efficiency on Chinese cabbage seedling to overcome salinity conditions. (Soy *et al.*, 2014).

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